

- (6, cont'd)
- 44.² The composition of claim 43,¹ wherein the polypeptide has an amino acid sequence according to SEQ ID NO:11.
- 45.³ The composition of claim 43,¹ wherein the composition comprises an enzyme activity with a Cre recombinase efficiency of about 16.8% per microgram of protein.
- 46.⁴ An isolated nucleic acid molecule comprising a coding region wherein the coding region encodes a glutathione-S-transferase-Cre-recombinase fusion polypeptide.
- 47.⁵ The nucleic acid molecule of claim 46,⁴ wherein the coding region comprises the nucleic acid sequence of SEQ ID NO:10.
- 48.⁶ The nucleic acid molecule of claim 46,⁴ wherein the isolated nucleic acid molecule is an expression vector.
- 49.⁷ The nucleic acid molecule of claim 46,⁴ wherein the coding region is operatively linked to a promoter effective to direct expression of a glutathione-S-transferase-Cre recombinase fusion polypeptide.
- 50.⁸ The nucleic acid molecule of claim 49,⁷ wherein the promoter is an inducible promoter.
- 51.⁹ The nucleic acid of claim 50,⁸ wherein the promoter is the *tac* promoter.
- 52.¹⁰ A host cell comprising the nucleic acid molecule of claim 46.⁴
- 53.¹¹ A host cell comprising the nucleic acid molecule of claim 49.⁷
- 54.¹² The host cell of claim 53,¹¹ wherein the host cell expresses a Cre recombinase activity.

- (61 cont'd)
- 55.¹³ The host cell of claim 53,¹¹ further defined as an E. coli cell.
- 56.¹⁴ A bacterial cell engineered to express a glutathione-S-transferase-Cre-recombinase fusion polypeptide.
- 57.¹⁵ The bacterial cell of claim 56,¹⁴ wherein the polypeptide has an amino acid sequence according to SEQ ID NO:11.
- 58.¹⁶ A method of producing a glutathione-S-transferase-Cre-recombinase fusion polypeptide comprising:

obtaining an expression vector comprising a coding region encoding a glutathione-S-transferase-Cre-recombinase fusion polypeptide operatively linked to a promoter;

transforming or transfecting the vector into a cell; and

growing the cell under conditions effective to express a glutathione-S-transferase-Cre-recombinase fusion polypeptide.
- 59.¹⁷ The method of claim 58,¹⁶ further comprising isolating the glutathione-S-transferase-Cre-recombinase fusion polypeptide.
- 60.¹⁸ The method of claim 59,¹⁷ wherein isolating the polypeptide comprises glutathione affinity chromatography.
- 61.¹⁹ A method of recombining nucleic acid segments, wherein each segment comprises a *lox* site specific recombinase site, the method comprising contacting the nucleic acid segments with a glutathione-S-transferase-Cre-recombinase fusion polypeptide.
- 62.²⁰ The method of claim 61,¹⁹ wherein the polypeptide has an amino acid sequence according to SEQ ID NO:11.

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63. A composition comprising a glutathione-S-transferase-Cre-recombinase fusion polypeptide and one or more nucleic acid molecules, wherein the nucleic acids comprise a site specific recombinase site.
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64. The composition of claim 63, wherein at least one of said nucleic acid molecules comprises a lox recombination site upstream in a 5' to 3' orientation from an amino acid encoding region.
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65. The composition of claim 63, wherein at least one of said nucleic acid molecules comprises a transcription regulatory element upstream in a 5' to 3' orientation of a lox recombinase site.
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66. The composition of claim 64 wherein the lox recombinase site is a loxP, loxP2, loxP3, loxP23, loxP511, loxB, loxC2, loxL, loxR, loxΔ86, loxΔ117, or loxH site.
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67. The composition of claim 65 wherein the lox recombinase site is a loxP, loxP2, loxP3, loxP23, loxP511, loxB, loxC2, loxL, loxR, loxΔ86, loxΔ117, or loxH site.
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68. The composition of claim 64, wherein the amino acid encoding region is a member of a nucleic acid library.

II. REMARKS

The claims in this preliminary amendment do not add new matter to the application and their entry is therefore respectfully requested. Support for the claims may be found throughout the Specification and at least in Example 3 found on page 47.

IV. CONCLUSION

Applicants respectfully submit that the present application and all claims are in condition for immediate allowance and early notice to such effect is earnestly solicited. If, in the opinion of the Examiner, a phone call may help expedite prosecution of this application, the Examiner is invited to contact the undersigned representative at (512) 542-8446.